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PCT/GB2004/002421

CLAIMS:

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1. A peptide consisting of or comprising an amino acid sequence selected from

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- a) $PX^{1}X^{2}X^{3}T$ [SEQ.ID.NO.:1];
- b) PSX4S [SEQ.ID.NO.:2];
- c) $QX^5X^6X^7Q$ [SEQ.ID.NO.:3];
- d) SX^8S [SEQ.ID.NO.:4],

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in which X^1 , X^2 and X^3 , which may be the same or different, each represents an amino acid residue;

X4 represents an amino acid residue; and

 X^5 and X^7 , which may be the same or different, each represents an amino acid residue, X^6 represents an amino acid residue

having an amide side chain; and

X⁸ represent an amino acid having an aliphatic side chain, with the proviso that a peptide comprising an amino acid sequence of SEQ.ID.NO.:1, 2, 3 OR 4 is not a naturally-

- 20 occurring full length protein.
 - 2. A peptide as claimed in claim 1 consisting of or comprising an amino acid sequence $PX^1X^2X^3T$ [SEQ.ID.NO.:1] in which X^2 represents N or L.

- 3. A peptide as claimed in claim 2, wherein X^2 represents L.
- 4. A peptide as claimed in claim 3, wherein X^1 represent S, 30 A or P.
 - 5. A peptide as claimed in claim 3 or claim 4, wherein X^3 represents S, K or T.
- 35 6. A peptide as claimed in any one of claims 3 to 5,

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wherein X1 represents A.

- 7. A peptide as claimed in any one of claims 3 to 6, wherein X^3 represents T.
- 8. A peptide as claimed in claim 3, consisting of or comprising the amino acid sequence PALKT.
- 9. A peptide as claimed in claim 1 or claim 2, wherein X^2 10 represents N.
 - 10. A peptide as claimed in claim 9, wherein X^1 represent S or P.
- 15 11. A peptide as claimed in claim 9 or claim 10, wherein X^3 represents S or T.
- 12. A peptide as claimed in claim 1, consisting of or comprising the amino acid sequence PALKT [SEQ.ID.NO.:6], PSNST [SEQ.ID.NO.8], or PPNTT [SEQ.ID.NO.:9].
 - 13. A peptide as claimed in any one of claims 1 to 12, having an A or V residue at the C-terminus.
- 14. A peptide as claimed in any one of claims 1 to 13, having an A, S or T residue at the N-terminus.
- 15. A peptide as claimed in claim 12 or claim 13, having the sequence STPPNTT [SEQ.ID.NO.:17], APSNSTA [SEQ.ID.NO.:15], and SPALKTV [SEQ.ID.NO.:16].
 - 16. A peptide as claimed in claim 1, consisting of or comprising an amino acid sequence PSX^4S [SEQ.ID.NO.:2], in which X^2 represents N or L.

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- 17. A peptide as claimed in claim 16, having an A or L residue at the N-terminus.
- 18. A peptide as claimed in claim 17, having the sequence LPSLS [SEQ.ID.NO.:22].
 - 19. A peptide as claimed in any one of claims 16 to 18, having one or more further residues at the N-terminus.
- 20. A peptide as claimed in claim 19, having the sequence MLPSLS SEQ.ID.NO.:23] or PMLPSLS [SEQ.ID.NO.:24].

- 21. A peptide as claimed in claim 1 consisting of or comprising an amino acid sequence $QX^5X^6X^7Q$ [SEQ.ID.NO.:3] in which, independently, X^6 represents an N or Q residue.
- 22. A peptide as claimed in claim 21, wherein independently, X^5 represents K or S and X^7 represents P or Y.
- 20 23. A peptide as claimed in claim 21 in which X^5 is K, X^6 is N and X^7 is P or X^5 is S, X^6 is Q and X^7 is Y.
- 24. A peptide as claimed in any one of claims 21 to 23 having an S or F residue at the N-terminus and/or an M or K residue at the C-terminus.
 - 25. A peptide as claimed in claim 24 having the sequence SQKNPQM [SEQ.ID.NO.:25] or FQSQYSQK [SEQ.ID.NO.:26].
- 26. A peptide as claimed in claim 1 consisting of or comprising an amino acid sequence SX^8S [SEQ.ID.NO.:4] in which X^8 represents L or I.
- 27. A peptide as claimed in claim 26, having, independently either or both of an A or P residue at the N-terminus and an

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M residue at the C-terminus.

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- 28. A peptide as claimed in claim 27, having one or more further residues at the N- terminus and/or C- terminus.
- 29. A peptide as claimed in claim 27 or claim 28, having the sequence PMLPSLS or MASISMK or a variant thereof in which one or more of the terminal residues are omitted.
- 10 30. A peptide as claimed in claim 1 and as describe in Table 1 herein.
- 31. A peptide as claimed in claim 1, having one or more amino acid residues at the N-terminus and/or at the C-terminus.
 - 32. A peptide as claimed in any one of claims 1 to 30 having up to 30 amino acids, for example, having up to 20 amino acids, for example, up to 12 amino acids, for example, having 7 amino acids.
 - 33. A peptide as claimed in any one of claims 1 to 32 comprising a cyclic region of amino acids.
- 25 34. A peptide as claimed in claim 33 wherein the peptide comprises two or more cysteine residues capable of forming one or more disulphide bond(s).
- 35. A peptide as claimed in any one of claims 1 to 34 but not subject to the proviso of claim 1, wherein the peptide is linked to a polycationic nucleic acid-binding component.
 - 36. A peptide as claimed in claim 35 wherein the polycationic nucleic acid-binding component is polyethylenimine or a dendrimer.

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37. A peptide as claimed in claim 35, wherein the polycationic nucleic acid-binding component is an oligopeptide comprising one or more cationic monomers.

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38. A peptide as claimed in claim 37, wherein the oligopeptide is an oligolysine, an oligoarginine, an oligohistidine, or a mixed oligomer comprising any combination of histidine, arginine and lysine residues.

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- 39. A peptide as claimed in claim 37 or claim 38, wherein the cationic oligopeptide has from 5 to 25 monomers, preferably from 10 to 20 monomers.
- 15 40. A peptide as claimed in claim 39, wherein the oligopeptide is oligolysine.
 - 41. A peptide as claimed in claim 40, wherein the oligolysine has from 14 to 18, for example, 6, monomers.

- 42. A peptide as claimed in any one of claims 35 to 41, wherein the peptide is linked to the polycationic nucleic acid-binding component via a spacer element.
- 43. A peptide as claimed in claim 42, wherein the spacer element is GG or GA or is longer and/or more hydrophobic than the dipeptide spacers GG (glycine-glycine) and GA (glycine-alanine).
- 30 44. A peptide as claimed in claim 42 or claim 43, wherein the spacer element is GA.
 - 45. A peptide as claimed in claim 42, wherein the spacer element is a chemical bond.

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46. A peptide as claimed in claim 36, wherein the peptide is linked to the polyethylenimine via a disulphide bond.

- 47. A peptide derivative of formula A-B-C wherein
 A is a polycationic nucleic acid-binding component,
 B is a spacer element, and
- C is a peptide as claimed in any one of claims 1 to 34, which peptide is not subject to the proviso of claim 1.
- 10 48. A peptide derivative as claimed in claim 47, wherein the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 41.
- 49. A peptide derivative as claimed in claim 47 or claim 15 48, wherein the spacer element is as defined in any one of claims 42 to 46.
- 50. A peptide derivative as claimed in claim 47, which derivative is in the form of a peptide as claimed in any one of claims 35 to 46.
- 51. A non-viral transfection mixture that comprises

 (ii) a lipid component,

 (iii) a polycationic nucleic acid-binding component, and

 (iv) a peptide as claimed in any one of claims 1 to 34,

 which peptide is not subject to the proviso of claim 1.
- 52. A mixture as claimed in claim 51, wherein the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 41.
 - 53. A mixture as claimed in claim 51, wherein components (iii) and (iv) are in the form of a peptide or peptide derivative as claimed in any one of claims 39 to 50.

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54. A mixture as claimed in any one of claims 51 to 53, wherein the lipid component is or comprises one or more lipids selected from cationic lipids and lipids having membrane destabilising or fusogenic properties.

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55. A mixture as claimed in claim 54, wherein the lipid component is or comprises the neutral lipid dioleyl phosphatidyl-ethanolamine (DOPE) or a lipid having similar membrane destabilising or fusogenic properties.

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56. A mixture as claimed in claim 54 or claim 55, wherein the lipid component is or comprises the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) or a lipid having similar cationic properties.

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- 57. A mixture as claimed in claim 56, wherein the lipid component is or comprises a mixture of DOPE and DOTMA, especially an equimolar mixture thereof.
- 20 58. A mixture as claimed in claim 57, wherein the ratio lipid component: peptide/polycationic nucleic acid-binding component is 0.75:4 by weight or 0.5 nmol:1.25 nmol on a molar basis.
- 59. A mixture as claimed in any one of claims 51 to 58, wherein the lipid component is or comprises 2,3-dioleyloxy-N-[2-(spermidinecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium-trifluoridoacetate (DOSPA) or a lipid having similar properties to those of DOSPA.

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60. A mixture as claimed in claim 59, wherein the lipid component is or comprises a mixture of DOPE and DOSPA, especially a mixture of one part by weight DOPE to 3 parts by weight DOSPA.

61. A mixture as claimed in any one of claims 51 to 53, wherein the lipid component is or comprises any one of more of the lipids of the general formula (I), (II) or (III):

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$$(R^{4})_{3}N^{+} - N^{+}(R^{3})_{3}$$

$$R^{1} - X^{1} - X^{2} - R^{2}$$
(I)

wherein

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- X^1 and X^2 are the same or different and are selected from -O-CH₂- and -O-C(O)-;
 - R¹ and R² are the same or different and are straight or branched, saturated or unsaturated C₇ to C₂₄ hydrocarbyl groups which are unsubstituted or substituted by one or more substituents selected from hydroxy, halogen and OR', wherein R' is a C₁ to C₆ hydrocarbyl group;
 - each R³ and each R⁴ is the same or different and is a straight or branched, saturated or unsaturated C₁ to C₁₀ hydrocarbyl group which is unsubstituted or substituted by one or more substituents selected from hydroxy, halogen, -OR', -C(O)OH, -CN, -NR'R", and -C(O)R" wherein R' and R" are the same or different and are C₁ to C₆ hydrocarbyl;

wherein

- X¹ and X² are the same or different and are as defined above;
- R¹ and R² are the same or different and are as defined above;

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 R^5 is $-N^+(R^3)_2-R^6$ wherein each R^3 is the same or different and is as defined above and R^6 is either:

- (a) $-[A-Y]_{-n}R^4$ wherein each Y is the same or different and is $-N^+(R^4)_2$ wherein R^4 is as defined above; each A is the same or different and is a C_{1-20} alkylene group which is unsubstituted or substituted by one or more substituents selected from hydroxy, halogen, -OR', -C(O)OH, -CN, -NR'R'', and -C(O)R'' wherein R' and R'' are the same or different and are C_{1-6} hydrocarbyl; and n is from 1 to 10, and
- R⁴ is as defined above; or

 (b) -[B-O]-mB-Q wherein:

 each B is the same or different and is a C₁₋₁₀

 alkylene group which is unsubstituted or substituted by one or more substituents selected from hydroxy, halogen, -OR', -C(O)OH, -CN, -NR'R" and -C(O)R" wherein R' and R" are the same or different and are C₁₋₆ hydrocarbyl;

 m is from 1 to 10; and
 Q is selected from -N⁺(R³)₃, -OH, -OR', -OC(O)R' and halogen, wherein R³ and R' are as defined above;

30 wherein:

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the Rs, which may be the same or different, are

- (a) H,
- (b) $-CH_2-N^+(R_2)^2-CH_2-CH_2-[Y-(CH_2)_p]_q-Z$, or
- $(c) CH_2 N^+ (R^4)_3,$
- with the proviso that one R is H and the other is group

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(b); or both groups R are groups (c); and wherein the Xs which may be the same or different, are OCH2 or O-C (O);

the R¹s, which may be the same or different, are saturated or unsaturated, C7 to C23 chains;

the R²s, which may be the same or different, are C1 to C6 saturated or unsaturated chains;

Y is NH, CH₂, O or N(acetyl);

Z is $O(C_1$ to $C_4)$, $OC(O)R^3$, $N^+R_3^4$, OH, F, Cl, Br or I where R^3 is Cl to C6 alkyl;

the R⁴s, which may be the same or different, are C1 to C6 chains;

n is from 2, 3 or 4; and

m is from 1 to 200 and where it is at least 2 the resulting repeating units may be the same or different.

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- 62. A mixture as claimed in any one of claims 51 to 61, which comprises a mixture the lipid component, a peptide as claimed in any one of claims 1 to 14, which peptide is not subject to the proviso of claim 1, and [K]₁₆ as the polycationic nucleic acid-binding component.
- 63. A mixture as claimed in claim 62, wherein the ratio lipid component:peptide/polycationic nucleic acid-binding component is 12:4:1 by weight.

- 64. A process for the production of a mixture as claimed in any one of claims 51 to 63, which comprises admixing components (ii), (iii) and (iv).
- 30 65. A non-viral transfection complex that comprises
 - (i) a nucleic acid,
 - (ii) a lipid component,
 - (iii) a polycationic nucleic acid-binding component, and
 - (iv) a peptide as claimed in any one of claims 1 to 34,
- which peptide is not subject to the proviso of claim 1.

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- 66. A complex as claimed in claim 65, wherein the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 41.
- 67. A mixture as claimed in claim 65, wherein components (iii) and (iv) are in the form of a peptide or peptide derivative as claimed in any one of claims 39 to 50.
- 10 68. A complex as claimed in any one of claims 65 to 67, wherein the lipid component is as defined in any one of claims 54 to 63.

- 69. A complex as claimed in any one of claims 65 to 68,
 wherein the nucleic acid component is or relates to nucleic
 acid sequence suitable for gene therapy, gene vaccination or
 anti-sense therapy, or encodes a desired protein.
- A complex as claimed in any one of claims 65 to 69, wherein the nucleic acid component is the coding sequence of 20 a protein or the cDNA copy or genomic version thereof, the latter including introns as well as exons, a regulatory upstream or downstream sequence of a gene, a sequence involved in repairing a gene or in homologous recombination, a short sequence contained in a plasmid, or another large 25 nucleic acid that mediates integration of plasmids or nucleic acids, for example, phage integrase or a "Sleeping Beauty" transposon, a DNA suitable for antisense regulation or as a transcription factor decoy, an oligonucleotide sequences useful as an adjuvant to boost a vaccine response, for 30 example, a CpG-rich sequence, or a small interfering RNA (siRNA).
- 71. A complex as claimed in claim 69 or claim 70, wherein transcriptional and/or translational control elements for the

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nucleic acid are provided and the nucleic acid is optionally packed in a phage or vector.

- 72. A complex as claimed in any one of claims 65 to 71, wherein the nucleic acid component is DNA.
 - 73. A complex as claimed in any one of claims 65 to 71, wherein the nucleic acid component is RNA.
- 74. A complex as claimed in claim 65, comprising a nucleic acid and a transfection mixture as claimed in any one of claims 51 to 63.
- 75. A complex as claimed in claim 74, wherein the nucleic acid is as claimed in any one of claims 69 to 73.

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- 76. A process for the production of a complex as claimed in any one of claims 65 to 73, which comprises admixing components (i), (ii), (iii) and (iv).
- 77. A process as claimed in claim 76, wherein the components are admixed in the following order: lipid component, peptide/polycationic nucleic acid-binding component, nucleic acid.
- 78. A process for producing a complex as claimed in claim 74 or claim 75, which comprises incorporating the nucleic acid with a mixture as claimed in any one of claims 51 to 63.
- 79. A complex as claimed in any one of claims 65 to 75, obtainable by a process as claimed in any one of claims 76 to 78.
- 80. A non-viral transfection complex that comprises

 (i) a nucleic acid,

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(iii) a polycationic nucleic acid-binding component, and (iv) a peptide as claimed in any one of claims 1 to 34, which peptide is not subject to the proviso of claim 1.

- 81. A complex as claimed in claim 80, wherein the nucleic acid is as defined in any one of claims 69 to 73, the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 41, and optionally the peptide is as defined in any one of claims 35 to 46, or is a peptide derivative as claimed in any one of claims 47 to 50.
 - 82. A process for the production of a complex as claimed in claim 80 or claim 81, which comprises admixing components (i), (iii) and (iv) in the following order:
- peptide/polycationic nucleic acid-binding component, nucleic acid.
 - 83. A complex as claimed in claim 80 or claim 81, obtainable by a process as claimed in claim 82.

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84. A viral vector that comprises a peptide as claimed in any one of claims 1 to 34.

- 85. A viral vector as claimed in claim 84, wherein the viral vector is a genetically engineered, replication-defective derivative of a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus (AAV), or a herpes simplex virus (HSV).
- 30 86. A viral vector as claimed in claim 85, wherein the viral vector is an adenovirus.
 - 87. A viral vector as claimed in claim 85, wherein the adenovirus is adenovirus type 5.

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- 88. A viral vector as claimed in any one of claims 84 to 87, wherein the peptide is incorporated in a protein of the viral capsid or coat.
- 89. A viral vector as claimed in claim 87 or claim 88, wherein the peptide is incorporated in the HI region of the fibre protein in the adenoviral capsid.
- 90. A viral vector as claimed in any one of claims 84 to 87, wherein the peptide forms a complex with the vector, the peptide comprising a cationic domain that is capable of binding electrostatically to the viral capsid or coat.
- 91. A viral vector as claimed in claim 90, wherein the
 15 peptide is as claimed in any one of claims 35 to 46, or is in
 the form of a peptide derivative as claimed in any one of
 claims 47 to 50.
- 92. A viral vector as claimed in any one of claims 84 to 87, wherein the peptide incorporated with the viral vector by means of an antibody that is capable of binding to the virus.
- 93. A viral vector as claimed in claim 92, wherein the antibody is a bispecific antibody capable of binding to the peptide and to the virus.
 - 94. A viral vector as claimed in claim 92, wherein the peptide and the antibody are in the form of a fusion protein.
- 95. A viral vector as claimed in any one of claims 92 to 94, wherein the antibody binds to an epitope on the viral capsid or coat.
- 96. A viral vector as claimed in any one of claims 92 to 95, wherein the antibody is of any antibody class, is an

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antigen-binding domain or domains, and/or is or is derived from a chimeric or humanised antibody.

- 97. A method of transfecting a cell with a nucleic acid, which comprises contacting the cell in vitro or in vivo with a transfection complex or a viral vector as claimed in any one of claims65 to 75, 79 to 81 and 83 to 96.
- 98. A pharmaceutical composition which comprises a transfection complex or a viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96, in admixture or conjunction with a pharmaceutically suitable carrier.
- 99. A method for the treatment or prophylaxis of a condition caused in human or in a non-human animal by a defect and/or a deficiency in a gene, which comprises administering a transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 to the human or to the non-human animal.
- 100. A method for therapeutic or prophylactic immunisation of a human or of a non-human animal, which comprises administering a transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 to the human or to the non-human animal.
 - 101. A method of anti-sense therapy, which comprises administering a transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 to a human or to a non-human animal.
 - 102. A transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 for use as a medicament or a vaccine.

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- 103. Use of a transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 for the manufacture of a medicament for the prophylaxis of a condition caused in a human or a non-human animal by a defect and/or a deficiency in a gene, or for therapeutic or prophylactic immunisation, or for anti-sense therapy.
- 104. A kit that comprises
 - (i) a nucleic acid,
- 10 (ii) a lipid component,
 - (iii) a polycationic nucleic acid-binding component, and (iv) a peptide as claimed in any one of claims 1 to 34, which peptide is not subject to the proviso of claim 1.
- 15 105. A kit that comprises

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- (i) a nucleic acid,
- (iii) a polycationic nucleic acid-binding component, and (iv) a peptide as claimed in any one of claims 1 to 34, which peptide is not subject to the proviso of claim 1.

106. A bispecific antibody that is capable of binding to a virus and to a peptide as claimed in any one of claims 1 to

- 107. A fusion protein that comprises a peptide as claimed in any one of claims 1 to 34, which peptide is not subject to the proviso of claim 1, and antibody that is capable of binding to a virus.
- 108. A method for identifying an siRNA, which comprises transfecting a cell that expresses a target gene with the siRNA and quantifying expression levels.